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Trace Mineral Dietary Interrelationships

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Introduction

The study of trace minerals in recent years has been receiving more attention, because frequently they are involved in nutritional disturbances. Present in the body only in tiny amounts, they are believed to be the links between the enzymes and the substances in the body on which the enzymes act. Study of these mineral trace elements is a study in interrelationships. We have learned that an element may have a specific function not tied to the function of another element. Calcium, for example, has a well-known function as part of the blood clotting mechanism. However, an animal's *utilization* of an element, such as calcium, cannot properly be evaluated without considering the availability, absorption and utilization of phosphorus, to choose only one of its interrelated elements.

Discovery of nutritional needs for mineral trace elements is a tribute to the skill of the analyst as well as of the nutritionist. That's because these minerals are needed in very small quantities by humans and animals and are involved in dietary interrelationships. As research progresses, it becomes clearer that to understand the role of any given trace element, we must understand not only the interrelationships between trace elements, but those between trace elements and the macro-elements.

The requirement for a given trace element may be modified by the presence or lack of another essential element. Under practical conditions, correcting a dietary deficiency or excess depends not only on the dietary level of the element in question, but on the level of its nutritionally related elements.

Copper and Iron

Copper is unique among the trace elements in the many metabolic functions it influences, and in its many interrelationships with other trace elements as well as with some of the macro-mineral elements (1). In demonstrating that copper is an essential element for mammals, workers at the University of Wisconsin found that the utilization of iron in hemoglobin formation was dependent upon an adequate intake of copper (2). This was quickly confirmed by other investigators. It is now known that a severe anemia develops in warm blooded animals with a copper deficiency, although copper does not appear to be a part of their hemoglobin molecule. This utilization of iron demonstrates the need to consider the joint nutrition of both iron and copper.

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The metabolic interrelationship of copper and iron does not appear to be identical in all species. In research with rats, dog, swine and rabbits, the acute anemia which develops in cases of copper deficiency is indistinguishable from an iron-deficiency anemia in the same species (3).

In cattle and in sheep suffering from a copper deficiency, there does not appear to be any interference with iron absorption. Iron absorption continues and even may be increased with a resultant extremely high accumulation of iron in the animal's tissues, such as the liver, even though there is a failure of normal hemoglobin formation. In these copper-deficient animals, the increasing levels of iron in the tissue do not appear to exert a control on iron absorption; levels as high as 4000 p.p.m. in fresh liver tissue have been reported under pasture conditions on organic soils (4, 5).

So close is the tie-up between iron and copper that with cattle pastured on peat soil, a suspicion of copper deficiency can be confirmed or eliminated by the determination of liver-iron. The level of iron in the liver has been shown a better indication of the nutritional status of copper than a determination of blood or liver-copper (6). Research reports from workers investigating copper deficiencies in cattle have suggested that the influence of copper on iron is most likely in the bone marrow or liver. In cattle with a dietary copper deficiency and concurrent high levels of iron in the liver tissue, the institution of copper therapy is accompanied by a very rapid conversion of iron into hemoglobin and a resultant remission of the anemia. Blood hemoglobin values have been observed to rise from values of 5 to 6 grams of hemoglobin per 100 milliliters of blood to values of 10 to 12 grams of hemoglobin per 100 milliliters of blood in periods of less than 10 days, with liver-iron values dropping concurrently from 3000 to 4000 p.p.m. to values of 300 to 400 p.p.m.

Investigations on copper deficiency in swine have suggested that copper may influence iron metabolism by exercising an effect upon iron absorption from the intestinal tract. In swine, a copper deficiency has resulted in depressed iron absorption, and the anemia which develops differs in a number of respects from the anemia which results from an iron deficiency of swine (7).

The exact role of copper is still an uncertainty, but whether it is as an enzyme activator, or an active intermediate in the formation of hemoglobin, or an essential for erythrocyte maturation, it is an essential key in iron metabolism. While differences exist in different species, they serve to emphasize the need for a consideration of iron and copper together in human nutrition, since there is a marked interdependence in animal metabolism.

Copper and Molybdenum

One of the most intriguing interrelationships which has been uncovered in research with animals has been that of copper and molybdenum. In addition, it has been shown recently that copper, molybdenum, and sulfate are interdependent. While copper deficiency has been produced in animals through the use

of purified or specialized diets, the natural occurrence of a copper deficiency had not been demonstrated until workers in Florida, England, the Netherlands, Australia, New Zealand, Czechoslovakia, Finland, and elsewhere were able to show that in ruminants one of the principal hazards encountered on peat and muck soils was the development of a nutritional anemia that could be corrected by the addition of copper to the feed (8).

The research and practical experience which led to the discovery of the role of molybdenum in copper nutrition of cattle and sheep grazed on pastures that produced abundant forage but failed to sustain the animals is an interesting story, chapters of which were written in many different countries by a number of different investigators. It emphasizes the fact that the requirements for one element, copper, could not be definitely determined until the influence of other elements, such as molybdenum, could be determined.

In the extensive peat soil areas of the world, native forage is often produced in quantities unmatched on other soil types. In sub-tropical areas, yields of dry matter often amount to 15 to 18 tons per acre per year. Casual observance of this material would indicate no reason why it could not be satisfactorily consumed and utilized by cattle and sheep. Yet, in Florida, for example, whenever animals were placed on pastures in these areas their initial rapid gain in weight was soon followed by an equal or greater loss. If the livestock owner did not remove his animals from these areas, losses were frequently extensive. As a result, many of these pastures were known and used as fattening pastures. The cattlemen had learned by sad experience that if cattle were left on these pastures for periods longer than three to four months losses would result, and the occasional animal left on such pastures for longer than six to seven months was not likely to survive.

The productive capacity of these areas led many farmers to attempt production of such crops as winter vegetables, sugarcane, potatoes, peanuts, and the common forages of improved pasture areas. The results appeared doomed to failure until research revealed that copper fertilizers would result in the yields for which these areas are now famous. Continuing research demonstrated that in addition to copper, such elements as manganese, zinc, phosphorus, potassium, and boron were also needed; but copper provided the keystone that opened these areas to exploitation.

The results of copper fertilization with plant programs led the cattlemen to attempt cattle-raising operations on pastures which had responded to the application of copper fertilizers. At times, and in some places, the cattle appeared to do well, and at other times disaster would strike and the typical symptoms of "muck sick" appeared. These symptoms included a severe anemia (often blamed on parasites), rapid loss of weight, diarrhea, achromatrichia, fragile bones, fetal abnormalities, and "quick death" of animals which had been in good condition. Most animals became unhealthy rapidly and if not removed from the pastures eventually became very weak and died.

One of the most characteristic changes in cattle on copper-deficient or molybdenum-toxic areas has been the loss of haircoat color. A similar change has been noted in black sheep where pigment fails to form as the copper deficiency develops. It was this early observed change, the fading of hair-coat color or "lime-hidedness" as it was called by cattlemen in Florida that suggested to the Florida workers a possible relationship between this condition and the copper deficiency of rats. It had been observed in hooded rats with a copper deficiency, that the black hair-coat color became gray. The common occurrence of an anemia in rats and cattle led to the suggestion that the changed hair-coat color was due to the cause of the anemia. This naturally led to experiments with copper supplemenation.

The first attempts at copper therapy met with quite erratic results. In some instances, the response was immediate and satisfactory, but in most instances the response was slow or non-existent. Part of the difficulty stemmed from calculating a copper requirement for cattle from results that were obtained with rats. Additional difficulties arose from the failure to understand the influence on copper requirements exerted by other elements in feed coming from the organic soil areas.

An amount of copper in the diet that completely satisfied requirements of cattle grazing on sandy-land pastures was quite inadequate to meet the needs of cattle on the peat or muck areas.

The knowledge that copper, given as a supplemental drench, would enable animals to overcome the apparent copper deficiency and the relatively short-time response secured from application of copper salts as fertilizer spotlighted an apparent relationship between the development of "muck sick" and the season of the year. Much better results were obtained when copper was applied as a fertilizer in the late spring, corresponding with the beginning of the rainy season, than were secured with copper fertilizer applications in the fall, the beginning of the dry season. It was this apparent seasonal effect in the response of animals to supplments of copper and to application of copper salts as fertilizer that led to an appreciation of the influence of other elements upon the nutritional requirement for copper.

The response of animals to copper was very obvious. The initiation of hair pigment formation immediately following copper therapy, the improved growth and performance of the calves, the rapid recovery of hemoglobin level, and the initiation of healing in bone fractures, all emphasized that copper supplements at comparatively high levels were successful. An apparent deficiency had existed, even though levels of copper in the feed far exceeded those required by animals in other areas and under other conditions.

Concurrent with the animal experimentation, chemical analyses of the soil, pasture forage, and of the native forage from peat soil has been made. It was discovered that while copper values in native forage ranged from approximately 1 p.p.m. to as high as 6 p.p.m., addition of fertilizer to the improved pastures rarely increased copper values above 25 p.p.m. in the dry matter immediately

following the application of copper salts. These copper values dropped to approximately 12 p.p.m. within a short period after application of the copper fertilizer. Subsequently, the copper values in the dry matter of the forage continued to drop and within six months were often as low as 7 to 8 p.p.m.

A situation thus existed, at least in Florida, where samples of the organic soil which had been fertilized with copper, in some instances for many years, could be shown to contain the equivalent of well over 100 pounds of copper sulfate per acre in the top six inches, but with the copper firmly absorbed on the soil particles. Forage samples from these areas usually did not exceed 10 p.p.m. of copper in the dry matter. In other areas, levels of 5 to 7 p.p.m. of copper in the dry matter promoted normal copper nutrition. Yet in the muck pasture areas, cattle required levels as high as 100 p.p.m. in the total diet to protect the animal from deficiency symptoms.

At this stage the report came from England on the relationship of molybdenum to the development of "teart" in cattle (9). The symptoms of "teart," as reported by the English workers, were so similar to those seen in the copper-deficiency area of the organic soils of Florida that molybdenum levels were investigated in forage samples from areas where the cattle were showing great difficulties. As detailed later, this led to the discovery that as the molybdenum content of the forage increased more copper was required to prevent the development of a copper deficiency. The analytical values for molybdenum in forage ranged all the way from less than 1 p.p.m. in the dry matter to more than 300 p.p.m. in samples taken from different areas and at different seasons of the year.

The investigation of the molybdenum content of the soils demonstrated that there were great variations, in general, paralleling the molybdenum content of forage, but it soon became apparent that the level of molybdenum in the pasture forage reflected the pH of the soil. For a given molybdenum content, the higher the pH, the higher the level of molybdenum in the plants.

As the investigation continued, it became increasingly apparent that the variation in molybdenum content of the plants was more closely tied to the rainfall than to any other climatic factor. Since these areas are under water control, during the dry season water pumped into the areas comes up from a limestone base, which means that these irrigation waters are quite rich in calcium, the net result being an increase in pH. Thus, during the dry season, there is a strong tendency for any molybdenum present in the soils to be released and made available to the plants. On the other hand, during periods of rainfall, water must be pumped out of these areas, and the natural decomposition of the organic matter in the soil tends to lower the pH and to render molybdenum less available. In a given pasture area, the molybdenum content of the forage may vary from levels of 10 p.p.m. to 25 p.p.m. within a comparatively short period of time. The variation in cattle response with season appeared to be due, in part, to the changing molybdenum level of the forage and was modified in varying degrees by the level of copper storage within the animal and the level of copper present in the forage.

It was quite obvious that a nutritional status of cattle, with respect to copper, could not be assessed without some knowledge of the molybdenum content of the ration. With levels of 5 p.p.m. of copper in the ration, a level adequate for normal nutrition under many circumstances, the presence of 1 to 2 p.p.m. of molybdenum would precipitate the development of a copper-deficiency syndrome in periods ranging from 3 months to a year depending upon the degree of copper storage and the age of the animal. With levels of molybdenum as high as 10 p.p.m. in the diet, copper levels of at least 25 p.p.m. became necessary to prevent the development of symptoms of copper deficiency. Levels of 100 p.p.m. appeared to control molybdenum levels as high as 150 p.p.m. in the diet (8).

With the inability to permanently increase copper levels in forage to values high enough to control molybdenum levels that occurred naturally in the forage, various methods of supplementing the copper intake of cattle had to be tried. In the Netherlands, a common practice has been to spray copper solutions on the forage a short time before the cattle graze the pasture. In Florida, the most common practice has been to drench animals showing deficiency symptoms with a solution of copper salts and to keep available to the animals mineral mixtures containing comparatively high levels of copper in an effort to satisfy the requirements of the cattle.

When inadequate amounts of copper are supplied to cattle on pastures high in molybdenum, the copper reserves are depleted when the feed contains an adequate level of sulfate. With this unusual depletion of the copper reserves, a sharp increase in iron content of the liver occurs which is followed by a progressive drop in the hemoglobin content of the blood. Concurrent with the drop in hemoglobin values, there has been a sharp alteration in the bone metabolism with a progressive drop in bone breaking strength. The change in bone appears to be due in part to a loss in total bone ash, but also in part due to a change in bone structure (8, 10).

The availability of radioactive isotopes, at about the time early studies were being carried out on the relationship of molybdenum to copper metabolism, permitted studies to be made with copper-64, molybdenum-99, and phosphorus-32. These isotope studies have underlined the importance of the effects of these elements upon each other in animal nutrition. They have also emphasized the species difference in utilization of these elements. In studies with cattle using radioactive phosphorus-32, high levels of molybdenum in the diet resulted in a marked increase of phosphorus excretion (11). Losses of phosphorus from the cow's body were shown to increase by from 100 to 200% when the diet contained less than 10 p.p.m. of copper and in excess of 50 p.p.m. of molybdenum.

The content of copper in the liver dropped rapidly in cattle when the diet contained levels of molybdenum of 40 to 80 p.p.m. Later work by Australian investigators demonstrated that this effect is noted only if the diet contains an adequate level of sulfate (12).

With the recognition that molybdenum had a very important nutritional effect upon copper availability and utilization by cattle and sheep, it was logical for

research workers to try and ascertain where and in what manner this effect was taking place within the animal's body. Because it was known that molybdenum is a very powerful complexing element, it was considered likely that molybdenum was tying up copper within the intestinal tract and rendering the copper unavailable to the animal. This would, in effect, create a deficiency by preventing copper in the diet from reaching the blood stream.

It was easily demonstrated that molybdenum would tie up copper, but with the use of radioactive isotopes it was equally well demonstrated that in the living animal this was not occurring in so far as the nutritional availability of copper and molybdenum was concerned (13).

The nutritional picture has been additionally confused by the observation of English workers that with pasture and growing forage a much more severe copper deficiency-molybdenum toxicity occurs than when the same pasture forage is fed as hay in dry lot. Furthermore, supplemental feeding of sodium or ammonium molybdate will not cause the changes in animals and in the apparent development of copper deficiency until levels considerably in excess of those occurring naturally are given to the animals. While with 10 p.p.m. of copper in the diet, intakes in the pasture forage of as much as 10 p.p.m. of molybdenum will create a severe copper deficiency syndrome, when molybdenum is fed in dry lot in the form of the molybdates, it may require several hundred p.p.m. of molybdenum in order to produce the same copper deficiency syndrome.

In the monogastric species, it is usual for the animals to receive somewhat higher levels of copper than may be the case with cattle and sheep on pasture; but in experimental work, levels of molybdenum considerably above those occurring in natural feeds have usually been required before copper deficiency symptoms are produced. In work with rats, levels of from 80 to 400 p.p.m. of molybdenum have been required before the copper deficiency syndrome develops even with levels of copper restricted to 1 to 2 p.p.m. in the diet (14). The somewhat confusing results with monogastric species and also those with cattle and sheep were given a big boost towards clarification with the discovery by Australian workers that adequate sulfate was required in the diet in order to insure the interaction of copper and molybdenum (12).

There is good evidence that the interaction between molybdenum and copper is mediated by inorganic sulfate and further that depending on the level of inorganic sulfate in the diet, the action of molybdenum upon copper may be quite different. When sulfate levels are low in the diet, high levels of molybdenum may result in an increased copper content of the liver and other tissues of the body. The molybdenum is very poorly excreted by the animal through the normal pathway of the urine and may interfere with copper excretion. On the other hand, with low but adequate levels of copper for normal nutrition, low levels of molybdenum, with sulfate in adequate amounts, will result in a depletion of copper and a rapid production of a copper deficiency syndrome.

Higher levels of molybdenum and high levels of sulfate with even sizeable intakes of copper will result in a rapid elimination of both molybdenum and

copper from the animal body, and depending upon the relative levels of copper and molybdenum, the production of a molybdenosis-copper deficiency syndrome. While many possible mechanisms for this interrelationship between copper, molybdenum and sulfate and between copper, molybdenum and phosphorus have been postulated, the clear demonstration of the mechanism of interaction remains to be satisfactorily defined.

Data on the effect of inorganic sulfate upon the relationships of copper to molybdenum have been supplemented by experimental work from the Regional Laboratory at Ithaca, New York. Workers there suggest the level of methionine in the diet may markedly influence the development of molybdenosis (15). The actual mechanism of this interrelationship also remains obscure. It may be pointed out, however, that the bone changes observed repeatedly when molybdenum levels have caused an interference with copper metabolism, and the observation that phosphorus elimination is greatly increased by the presence of molybdenum in the diet, suggest that perhaps the sulfate ion or the sulfur-containing molecule may be responsible for this influence upon phosphorus metabolism. The close relationship between sulfur and bone formation suggests that such an interrelationship is possible.

The multiple interrelationships which exist between copper, sulfur, molybdenum and phosphorus may also include other mineral elements. In cattle, there seems to be a specific interference with the formation of vitamin B_{12} by levels of molybdenum which interfere with copper utilization. The mechanism appears to be concerned with rumen microflora and the metabolism of these organisms. With levels of approximately 100 p.p.m. of molybdenum in the diet, vitamin B_{12} synthesis in the rumen of cattle is depressed and may actually be halted. However, with increased cobalt levels in the diet and molybdenum maintained at 100 p.p.m., there appears to be a renewal of vitamin B_{12} synthesis. This would point to a microbiological defect caused by molybdenum when cobalt levels are near the minimum requirement level (16).

Copper and Zinc

Another element, whose metabolic role is greatly influenced by other mineral elements in the diet, is zinc. Workers in the Netherlands and in the United States have noted, in studies of liver metabolism, an apparent inverse relationship between copper and zinc (17, 18). In fact, efforts have been made to cause a copper deficiency syndrome by increasing the levels of zinc, and conversely, efforts have been made to produce a zinc deficiency by increasing copper levels in the liver. A high level of copper in the liver, such as may occur with copper toxicity, will result in an almost complete elimination of zinc from liver tissue. The mechanism of this interrelationship is obscure but apparently is within the animal body as opposed to the digestive tract.

This is illustrated by experiments at the Florida Station which show that increasing levels of zinc in the diet do not necessarily mean an increased level of zinc in the liver with a concurrent change in copper level. Increasing the

levels of copper in the diet do not necessarily mean a decreased level of zinc in the liver. Absorption from the diet is necessary before the interaction is apparent within the body tissues. If copper is absorbed and is deposited in the liver, as occurs under usual conditions in ruminants when excess copper sulfate is included in the diet, or in monogastric animals under conditions favoring absorption, then the zinc level in the liver will be depressed and symptoms of zinc deficiency will be observed. These symptoms include failure of the genital tract to develop in females. The possibility that the interaction of copper and zinc is mediated by a third factor is a likely possibility but has not as yet been demonstrated.

Zinc and Calcium

Probably the most publicized interrelationship of zinc with another element is that of zinc and calcium. In a search for the cause of parakeratosis in swine, workers in Alabama (19), Michigan (20), Wisconsin (21), and elsewhere have demonstrated that this condition is precipitated either by high levels of calcium or low levels of zinc. At least, under some conditions, parakeratosis can be corrected by increased zinc levels in the diet or by lowering calcium intakes. The results obtained by workers in various areas suggest that the interaction between zinc and calcium may very well be within the intestinal tract. However, the results obtained at the hands of different investigators using quite different levels suggest that there may be other factors involved in this interaction which remain to be demonstrated.

SUMMARY

The studies reported here demonstrate the complex interrelationships of trace mineral elements in animal nutrition — interrelationships that make it difficult if not impossible to evaluate dietary requirement of isolated components of the nutritional mixture called a diet unless such relationships are considered. As they function in a living body, these elements interset in metabolic reactions so as to spare or increase the need of other elements. In many cases, their nutritional behavior if profoundly affected by the presence of other trace elements and macro-elements.

Nutrition investigators have traditionally determined animal nutrient requirements as if the elements stood alone. The newer knowledge of nutritional interrelationships throws new light on the conflicts and differences in research findings that have often brought much confusion. Digestion, absorption, utilization and excretion of mineral elements are influenced by the form and level of other elements in the diet. These facts should be considered by physicians, nutritionists, biologists, veterinarians and husbandmen.

In the light of the trace minerals nutritional effects when in combination, the danger of "shotgun" supplement dosing is evident. Such mixtures are widely used in an effort to insure that nutritional requirements are adequately met. They

may contain more-than-adequate amounts of the different mineral elements, based on separately-computed requirement figures. Such "shotgun" mixtures are particularly dangerous when used indiscriminately and over considerable periods of time. The studies on relationship of calcium with zinc is a practical example, showing how increasing the calcium levels to insure "adequate" intakes resulted in a costly problem to swine husbandmen, because they did not understand that a change might occur in zinc requirements.

Because the interrelationships of mineral elements have such a bearing on nutritional requirements, widely varying results have been obtained by different investigators. Understanding of these differences may provide the key to better nutrition for both humans and animals, under the varying dietary conditions in the world today.

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